Pregnane and Pregnane Glycosides from *Trachelospermum liukuense*¹⁾

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Cortexone and three new bisdesmosidic glycosides of teikagenin were isolated from *Trachelospermum liukuense*, together with five glycosides already known in *Trachelospermum asiaticum*. The new glycosides have D-digitalose linked to the 20-OH as well as to the 3-OH.

Keywords *Trachelospermum*; Apocynaceae; pregnane; cortexone; teikagenin; 20S,5 α -pregn-6-en-3 β ,17 α ,20-triol; teikagenin 3,20-bis-O-digitaloside; teikaside

In the course of studies on the constituents of genus *Trachelospermum*, we have investigated the pregnane glycosides,^{1–3)} lignans⁴⁾ and triterpenoids^{5,6)} from *Trachelospermum asiaticum* NAKAI. The bisdesmosidic glycosides of 20S,5 α -pregn-6-en-3 β ,17 α ,20-triol (teikagenin) with a β -D-digitalosyl or 4-O-acetyl-L-sarmentosyl-(1 $\rightarrow$ 4)- β -D-digitalosyl group at the 3-OH and 2,6-dideoxy-3-O-methylhexoses at the 20-OH (teikasides) were isolated and their structures were established.

The species indigenous to the Ryukyu Islands is classified as *Trachelospermum liukuense* HATSUSIMA. In order to compare the constituents of the two species, the pregnane glycosides from *T. liukuense* were investigated. This paper deals with the isolation and structure identification of cortexone and bisdesmosidic glycosides of teikagenin.

When the MeOH percolate was partitioned with benzene, CHCl₃ and then BuOH in the usual manner, compound **1**, showing less polar behavior on thin layer chromatography (TLC), was isolated from the benzene extract. Based on the M⁺ peak of **1** at *m/z* 330.220 (C₂₁H₃₀O₃) in the electron impact (EI) mass spectrum (MS) and that of **1**-monoacetate (**1a**) at *m/z* 372.227 (C₂₃H₃₂O₄), **1** seemed to be a pregnane. In the proton nuclear magnetic resonance (¹H-NMR) spectrum of **1**, methylene protons in a primary carbinol group (δ 4.45 and 4.53, doublet each, *J* = 19 Hz) and one olefinic proton (δ 5.86) were observed together with two tertiary methyl groups, and **1** was considered to be cortexone (deoxycorticosterone). The structure of **1** was confirmed by carbon-13 nuclear magnetic resonance (¹³C-NMR) spectral comparisons with authentic cortexone and

cortexone acetate, as well as by TLC and high-performance liquid chromatography (HPLC).

From the CHCl₃ extract, three new compounds (**2–4**) were isolated together with the known teikagenin bisdesmosidic glycosides (**5–9**), by chromatographies on a polystyrene (MCI gel) column, an octadecyl silica (ODS)

TABLE I. ¹H Chemical Shifts of Compounds **2–4**, δ (ppm) from TMS in Pyridine-d₅ (*J*/Hz in Parentheses)

	2	3	4
H-3 α	3.98 (m)	3.93 ^{c)} (m)	3.95 ^{e)} (m)
H-6	5.54 (br d, 10)	5.53 (br d, 10)	5.55 (br d, 10)
H-7	5.33 (br d, 10)	5.32 (br d, 10)	5.33 (br d, 10)
H-18, 19	0.75, 0.78	0.72, 0.77	0.75, 0.76
H-20	4.17 (q, 6)	4.17 (q, 6)	4.11 (q, 6)
H-21	1.63 (d, 6)	1.63 (d, 6)	1.61 (d, 6)
3-O-Sugars	(digit.) ^{a)}	(digit.-1)	(digit.-2)
H-1	4.84 (d, 8)	4.76 ^{d)} (d, 8)	4.82 ^{e)} (d, 8)
H-2	4.34 (dd, 8, 9)	4.34 (dd, 8, 9)	4.32 (dd, 8, 9)
H-3	3.53 (dd, 9, 3)	3.61 (dd, 9, 3)	3.46 (dd, 9, 3)
H-4	4.08 (d, 3)	4.21 ^{d)} (d, 3)	4.01 (d, 3)
H-5	3.82 (q, 6)	3.75–3.85 (q, 6)	3.81 (q, 6)
H-6	1.59 (d, 6)	1.56 ^{b)} (d, 6)	1.60 ^{b)} (d, 6)
3-O-Me	3.60	3.73	3.57
20-O-Sugars	(digit.) ^{a)}	(digit.)	(digit.)
H-1	4.82 (d, 8)	4.85 (d, 8)	4.78 (d, 8)
H-2	4.31 (dd, 8, 9)	4.32 (dd, 8, 9)	4.38 (dd, 8, 9)
H-3	3.50 (dd, 9, 3)	3.50 (dd, 9, 3)	3.55 (dd, 9, 3)
H-4	4.07 (d, 3)	4.07 (d, 3)	4.32 ^{g)} (d, 3)
H-5	3.80 (q, 6)	3.75–3.85 (q, 6)	3.77 (q, 6)
H-6	1.58 (d, 6)	1.58 (d, 6)	1.58 (d, 6)
3-O-Me	3.56	3.56	3.65

a, b) Signal assignments marked a) or b) may be reversed. c–g) NOE was observed between the signals marked c–f) or g).

Chart 1

TABLE II. ^{13}C Chemical Shifts of Compounds 1–4, δ (ppm) from TMS in Pyridine- d_5

	1	2	3 ^{a)}	4 ^{a)}
C-1	34.3 ^{b)}	34.9	34.8	34.8
C-2	32.6 ^{c)}	30.0	29.9	30.0
C-3	198.2	77.7	77.7	77.6
C-4	124.1	33.0	32.8	32.9
C-5	170.2	45.1	45.1	45.1
C-6	35.8 ^{b)}	129.4	129.4	129.4
C-7	32.1 ^{c)}	131.1	131.0	131.1
C-8	35.4	38.2	38.2	38.2
C-9	56.0	52.7	52.6	52.6
C-10	38.6	34.5	34.5	34.5
C-11	21.1	20.9	20.9	20.9
C-12	38.5	38.4	38.4	38.4
C-13	44.4	47.1	47.1	47.1
C-14	53.6	49.1	49.0	49.0
C-15	24.6	23.6	23.6	23.5
C-16	23.2	31.9	31.9	31.9
C-17	58.7	85.3	85.3	85.2
C-18	13.5	11.4	11.4	11.1
C-19	17.0	14.5	14.5	14.5
C-20	210.8	82.7	82.7	83.1
C-21	70.1	18.0	18.0	18.1
3-O-Sugars		(digit.) ^{d)}	(digit.-1)	(digit.-2)
C-1		102.7	102.5	106.1
C-2		70.9	71.5	71.9
C-3		85.0	85.4	84.6
C-4		68.7	77.7	68.8
C-5		71.1	70.4	71.3
C-6		17.4	17.1 ^{e)}	17.7 ^{e)}
-OMe		57.2	59.0	57.3 ^{f)}
20-O-Sugars		(digit.) ^{d)}	(digit.)	(digit.) (glc.)
C-1		105.7	105.7	105.8
C-2		71.0	71.3	71.8
C-3		84.8	84.8	85.4
C-4		68.5	68.5	76.2
C-5		71.3	71.1	70.5
C-6		17.3	17.3 ^{e)}	17.6
-OMe		57.1	57.1 ^{f)}	58.8

a) Signal assignments were done based on the 2-dimensional (2D) NMR (^1H - ^{13}C COSY) spectra. b–f) Signal assignments marked b–f) may be reversed.

column and a silica gel column.

Compound **2** showed the characteristic signals due to 21-methyl protons (δ 1.63, d), a 20-methine proton (δ 4.17, q) and 6,7-olefinic protons (δ 5.33, d and δ 5.44, d) of teikagenin. Since proton signals assignable to two mol of D-digitalose were observed and the molecular formula was suggested to be $\text{C}_{35}\text{H}_{58}\text{O}_{11}$ by fast atom bombardment (FAB) MS, **2** was considered to be a didigitaloside of teikagenin. In the ^{13}C -NMR spectrum, all carbon signals due to two mol of digitalose were observed without a glycosylation shift, and the C-3 and the C-20 signals of teikagenin were shifted downfield. The structure of **2** was thus determined to be teikagenin 3,20-bis-O- β -D-digitaloside, and **2** was named teikaside AL-Ic.

On the basis of the $\text{M}^- - 1$ peak at m/z 813 in the negative FAB-MS, **3** was suggested to have one additional 6-deoxy-3-O-methylhexose as compared with **2**. All proton signals due to the three component sugars were assignable in the ^1H - ^1H correlation spectroscopy (COSY) measurement, starting with three anomeric proton signals at δ 4.76, 4.86 and 4.85, and the coupling constants of H-1–H-6 indicated the sugars to be D-digitalose. The H-4 signal and neighboring H-3 and 3-O- CH_3 signals were shifted down-

field in one of the digitalose residues, suggesting the presence of a 1 $\rightarrow$ 4 linked digitalosyl moiety. The chemical shifts in the ^{13}C -NMR spectrum were consistent with the ^1H -NMR assignments, showing the presence of two terminal digitalose residues, and a downfield shift of the C-4 signal in the remaining digitalose.

In order to determine the location of the digitalobiose, the difference nuclear Overhauser effect (NOE) measurement was applied. When the signal of H-3 α in the aglycone (δ 3.93) was irradiated, NOE was observed at the anomeric proton signal at δ 4.76. The H-4 signal at δ 4.21 in the same digitalose showed NOE with the second anomeric proton signal at δ 4.86. The structure of **3** was therefore determined to be teikagenin 3-O- β -D-digitalosyl-(1 $\rightarrow$ 4)- β -D-digitalosyl-20-O- β -D-digitaloside and **3** was named teikaside BL-Ic.

The molecular formula of **4** was suggested to be $\text{C}_{41}\text{H}_{68}\text{O}_{16}$ by the $\text{M}^- - 1$ peak at m/z 815 in the negative FAB-MS. Three anomeric proton signals were observed as doublets ($J=8$ Hz), and two 6-methyl proton signals and two methoxyl proton signals were observed besides the signals due to teikagenin, indicating **4** to be a teikagenin triside composed of two 6-deoxy-3-O-methylhexoses, possibly digitalose, and one hexose. All proton signals in the component sugars were assignable based on the ^1H - ^1H COSY results, and the sugars were determined to be two digitalose and one glucose. Since glycosylation shifts were observed at C-3 and C-20 of teikagenin and at C-4 of one of the digitalose residues, the glucose seemed to be linked to 4-OH of one digitalose. The location of the glucose was finally determined by the difference NOE procedure in the same manner as applied in the case of **3**. While the H-3 α signal of the aglycone at δ 3.95 showed NOE with one of the anomeric proton signals at δ 4.82, the H-4 signal of the same digitalose at δ 4.08 showed NOE only with the methyl protons of the neighboring methoxyl group, indicating that one digitalose is linked to the 3-OH of teikagenin. When the anomeric proton of the glucose at δ 5.13 was irradiated, NOE was observed at H-4 (δ 4.32) and the methoxyl proton signals in the remaining digitalose. On irradiation of the H-4 signal of this digitalose at δ 4.32, the anomeric proton signal of the glucose showed NOE. The glucose was therefore assigned to the 4-OH position in the digitalose linked to the C-20 hydroxyl group. Compound **4** was determined to be teikagenin 3-O- β -D-digitalosyl-20-O- β -D-glucosyl(1 $\rightarrow$ 4)- β -D-digitaloside and was named teikaside AL-IIc.

Compounds **5**–**9** were identified as teikagenin 3-O- β -D-digitaloside, and teikasides A-Ia, A-Ib, A-IIa and A-IIc, respectively in comparison with authentic samples obtained from *T. asiaticum*.²⁾

Neither cortexone nor teikagenin 20-O-digitaloside has been isolated from *T. asiaticum*. It should be noted that cortexone is a defense substance of water beetles, *Dysticus marginalis*.⁷⁾

Experimental

Specific rotation was recorded with a JASCO DIP 360 polarimeter. ^1H - and ^{13}C -NMR spectra were recorded on a JEOL GX-400 spectrometer in pyridine- d_5 . Chemical shifts are given in δ values referred to internal tetramethylsilane (TMS), and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=doublet of doublets, brs=broad singlet, brd=broad doublet. The following solvent systems were applied for silica gel column chromatography and

TLC: solvent 1, benzene–acetone; solvent 2, EtOAc–hexane; solvent 3, CHCl_3 –MeOH– H_2O (bottom layer); solvent 4, EtOAc–MeOH– H_2O (top layer or homogeneous layer); solvent 5, CH_3CN – H_2O . Detection of the spots was carried out by spraying diluted H_2SO_4 onto the TLC plates followed by heating the plates. HPLC was carried out on a Waters ALC 200 equipped with Radial Pack C_{18} eluted with solvent 5.

Extraction Air-dried plants of *Trachelospermum liukiense* (15 kg) collected at Tanegashima in January, 1987, were powdered and percolated with MeOH. The MeOH percolate (60 l) was concentrated to 5 l *in vacuo*, and partitioned with benzene (extract, 12 g) and CHCl_3 (150 g), then the water layer was concentrated *in vacuo*. The concentrated solution was then partitioned with BuOH (588 g).

Cortexone (1) The benzene extract was chromatographed on silica gel columns with solvent 1 (10:1), solvent 3 (7:1:3) and then solvent 2 (3:1–2:1) to yield a solid (1), showing a single spot on TLC (solvent 1). The solid was further purified by a reversed-phase column chromatography on an ODS column with solvent 5 (40%) to give pure 1 (12 mg) as a solid, $[\alpha]_D^{26} + 151.6^\circ$ ($c=0.095$, MeOH). UV $\lambda_{\text{max}}^{\text{MeOH}}$ nm (ϵ): 238 (8600). EI-MS m/z : 330.220 (Calcd for $\text{C}_{21}\text{H}_{30}\text{O}_3$: 330.220). $^1\text{H-NMR}$ δ : 0.68, 0.99 (3H each, s, H-18, H-19), 4.45, 4.53 (1H each, d, $J=19$ Hz, H-21a, b), 5.86 (1H, d, $J=2$ Hz, H-4). On TLC with solvents 1 and 2, the same R_f values were obtained in a parallel run with authentic cortexone. HPLC t_R 11.6 min (solvent 5, 50%). Upon usual acetylation of 1 with pyridine and Ac_2O at room temperature, 1-monoacetate was obtained and crystallized from hexane–ether to give prisms, mp 150–154°C, EI-MS m/z : 372.227 (Calcd for $\text{C}_{23}\text{H}_{32}\text{O}_4$: 372.230). On admixture with authentic cortexone acetate, no melting point depression was observed. In parallel with authentic cortexone acetate, the same R_f values were obtained in TLC with solvents 1 and 2.

Isolation of Teikasides The CHCl_3 extract was subjected to MCI-gel chromatography (elution with 0%–80% MeOH), and the eluate with 40–

60% MeOH was then fractionated by silica gel column chromatographies with solvent 3 (7:1:1) and solvent 4 (4:1:5–4:1:4) to afford 2–9 as solids. Yields: 2 (20 mg), 3 (6 mg), 4 (16 mg), 5 (80 mg), 6 (36 mg), 7 (5 mg), 8 (18 mg), 9 (6 mg). Compounds 5–9 were identified as teikagenin 3- O - β -D-digitaloside, and teikasides A-Ia, A-Ib, A-IIa and A-IIc, respectively, by comparison with the authentic teikasides on TLC and/or HPLC.

Teikaside AL-Ic (2): A solid, $[\alpha]_D^{28} - 86.5^\circ$ ($c=1.00$, MeOH). FAB-MS m/z : 677.387 (Calcd for $\text{C}_{35}\text{H}_{58}\text{O}_{11} + \text{Na}$: 677.388).

Teikaside BL-Ic (3): A solid, $[\alpha]_D^{28} - 79.1^\circ$ ($c=0.35$, MeOH). Negative FAB-MS m/z : 813 ($\text{M}^- - 1$), 653 ($\text{M}^- - \text{digit.} - 1$), 491, 377.

Teikaside AL-IIId (4): A solid, $[\alpha]_D^{29} - 74.1^\circ$ ($c=0.80$, MeOH). Negative FAB-MS m/z : 815 ($\text{M}^- - 1$), 653 ($\text{M}^- - \text{glc.}$), 491, 371.

Acknowledgements We thank Messrs. K. Aoi and K. Kaburagi of Tanegashima Experiment Station of Medicinal Plants, National Institute of Hygienic Sciences, for the collection of *Trachelospermum liukiense*. Our thanks are also due to Misses Y. Iwase and S. Hachiyama of Fukuoka University for NMR and MS measurements.

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